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In vitro antibacterial activity of crude ethanolic extract from the leaves of *Origanum vulgare*, against *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922

Actividad antibacteriana *in vitro* de extracto etanólico crudo de las hojas de *Origanum vulgare*, frente *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853 y *Escherichia coli* ATCC 25922

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Data of the Article

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Resumen

El presente trabajo cuyo objetivo fue evaluar la actividad antibacteriana *in vitro* del extracto etanólico de hojas de *Origanum vulgare* frente a *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853 y *Escherichia coli* ATCC 25922. El estudio fue experimental, se emplearon 72 unidades experimentales, constituidas por un tipo de extracto etanólico tres concentraciones, tres especies bacterianas y 8 repeticiones por grupo experimental. A través del método de dilución doble seriada se determinaron las diferentes concentraciones, para la actividad antibacteriana se empleó el método de difusión en pozo. Se emplearon concentraciones de 80, 40 y 20 mg/mL. El extracto etanólico presentó actividad antibacteriana *in vitro*, con un promedio del tamaño los halos de inhibición para *S. aureus* de 21.64, 15.24 y 11.45 mm, *P. aeruginosa* 13.31, 12.27 y 7.35 mm, *E. coli* de 12.5, 11.40 y 10.6 mm para las diferentes concentraciones. Se concluye que el extracto etanólico de *O. vulgare* tienen capacidad antibacteriana sobre *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853 y *E. coli* ATCC 25922.

Abstract

The present work whose objective was to evaluate the in vitro antibacterial activity of the ethanolic extract of *Origanum vulgare* leaves against *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922. The study was experimental, 72 experimental units were used, consisting of a type of ethanolic extract three concentrations, three bacterial species and eight repetitions per experimental group. Through the serial double dilution method, the different concentrations were determined, for the antibacterial activity, the well diffusion method was used. Concentrations of 80 mg/mL, 40 mg/mL and 20 mg/mL were used. The ethanolic extract showed antibacterial activity in vitro, with an average size of the inhibition halos for *S. aureus* of 21.64, 15.24 and 11.45 mm, *P. aeruginosa* 13.31, 12.27 and 7.35 mm, *E. coli* 12.5, 11.40 and 10.6 mm for the different concentrations. It is concluded that the ethanolic extract of *O. vulgare* has antibacterial capacity on *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922.

Introduction

The appearance of resistant microorganisms (MR), either through mutations or the acquisition of mobile genetic elements that carry resistance genes, can take place independently of the presence of antibacterial agents, being a threat to global health[1].

In the last decades, due to the evolution of bacteria and the abuse of antibiotics, the resistance of *Staphylococcus aureus* has gradually increased the rate of infection by multidrug-resistant *S. aureus* (MRSA) and anti-infective clinical treatment has become more difficult[2]. There are even studies that indicate that 63% of MRSA isolates produce biofilms with a resistance response to two antibiotics such as erythromycin and clindamycin, being strains with biofilm-forming genes[3].

The findings have demonstrated the existence of antibiotic-resistant microbes that are largely present in the Intensive Care Unit (ICU), with *Pseudomonas aeruginosa* being responsible for a wide range of infections acquired in the ICU in critically ill patients[4], being reported the Intrinsic resistance or the acquisition of chromosomal mutations through the acquisition of resistance genes against penicillins, cephalosporins, monobactams, carbapenems including aminoglycosides and fluoroquinolones[5].

On the other hand, uropathogenic *Escherichia coli* (UPEC) is responsible for urinary tract infections, which has an arsenal of virulence genes, the most frequent being the *fimH* gene, followed by the genes: *aer*, *hly*, *pap*, *cnf*, *sfa* and *afa* that contribute to their ability to overcome different defense mechanisms and cause disease[6], in addition to isolates of *E. coli* there is variation with respect to the susceptibility of antibiotics such as cefotaxime, nitrofurantoin, cefuroxime, ceftazidime, nalidixic acid, ciprofloxacin and even the prevalence of *E. coli* that produces extended spectrum β-lactamases (ESBL) has increased significantly[7,8].

The antibacterial and antioxidant properties of oregano have been attributed mainly to carvacrol and thymol, which are the main components of its essential oil that cause structural and functional alterations in the cell membrane[9-11], Also in a study, the extracts of leaves of *O. vulgare* has also been tested for its bactericidal activity against different important pathogens for fish aquaculture[12-14], with findings of activity against bacteria that cause periodontal diseases[15,16], it is even used in the storage stability of meat and prevent its decomposition[17, 18].

The antimicrobial activity of *O. vulgare* extracts has a predilection for Gram-positive bacteria such as *S. aureus*, but varies against Gram-negative bacteria such as *E. coli*, *K. pneumoniae*, *P. aeruginosa*[19-21] and even depending on the solvent such as ethanol and methanol allow the isolation of bioactive compounds of great interest in their antioxidant, antibacterial and antifungal action[22-24], as well as their finding with antiviral activity[25].

Since the antibacterial effect of the ethanolic extract is possible, it may present variations according to its concentrations, it was developed for the present investigation whose objective was to evaluate the in vitro antibacterial effect of the ethanolic extract of the leaves of *O. vulgare* against *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922.

Materials and methods

An in vitro study was carried out with an experimental design of increasing stimulus[26], with ethanolic extract (EE) of *O. vulgare*, 3 concentrations of each extract and with 8 repetitions, ethanol and negative control (NC) water were used as a positive control (PC). sterile distilled.

From plant material. Specimens of *O. vulgare* with branches, leaves and flowers[27] were collected from neighboring crops in the Chiclayo district, Lambayeque Region, Peru, of which two specimens were transported to the herbarium of the Faculty of Biological Sciences of the National University Pedro Ruiz Gallo for its identification, the process of disinfection of the leaves with 95º GL alcohol was then started. Subsequently, the leaves were dried using an oven at a temperature of 50 °C for eight hours. Afterwards, 500 g of leaves went to the milling stage until obtaining powdered material.

Preparation of the ethanolic extract of O. vulgare. For the preparation of EE[28], 40 g of *O. vulgare* powder were macerated and 200 mL of absolute ethanol were added, in a hermetic amber glass bottle, the bottle was shaken daily for a week at room temperature, the product was filtered with paper Whatmann filter No. 40, a residue-free extract was obtained, the filtrate was concentrated in Sohxlet until dryness, 2 g of dry residue was stored in a refrigerator at 2 °C in an amber glass bottle. For the concentrations, 2 g of dry residue was dissolved in 25 mL of absolute ethanol, obtaining a stock solution of 80 mg/mL, serial double dilutions were made, obtaining concentrations of 40 mg/mL and 20 mg/mL.

Inoculum preparation of S aureus ATCC 29213, *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922. They were purchased from the American Type Culture Collection Culti-Loops™ Thermo Scientific™. According to the guidelines of the Clinical and Laboratory Standards Institute[29], 3 to 5 colonies were selected from a culture on a Mueller-Hinton agar plate after 24 h. The upper part of each colony was touched with a loop and transferred to a tube with 4-5 mL of Müller-Hinton broth and with the help of the densitometer (DEN-1B) turbidity was measured with absorbances of 0.08-0.1 for bacteria equivalent to 0.5 of the McFarland[30] standard, obtaining a bacterial suspension resulting from 1 to 2 x 10^8 (CFU/mL). In an optimal time span of 15 min after adjusting the turbidity of the inoculum suspension, with a cotton swab it was inoculated over the entire surface of a Mueller-Hinton agar plate.

Antibacterial activity of O. vulgare extracts. Through the well diffusion method[30], on the seeded plates, 4 perforations of 6 mm in diameter were made, with a punch and 50 µL of oregano extracts were placed in each well, as PC used ethanol and NC sterile distilled water Afterwards, they were sealed with parafilm, incubated at 37 °C, for a period of 24 h, then the inhibition diameter was measured.

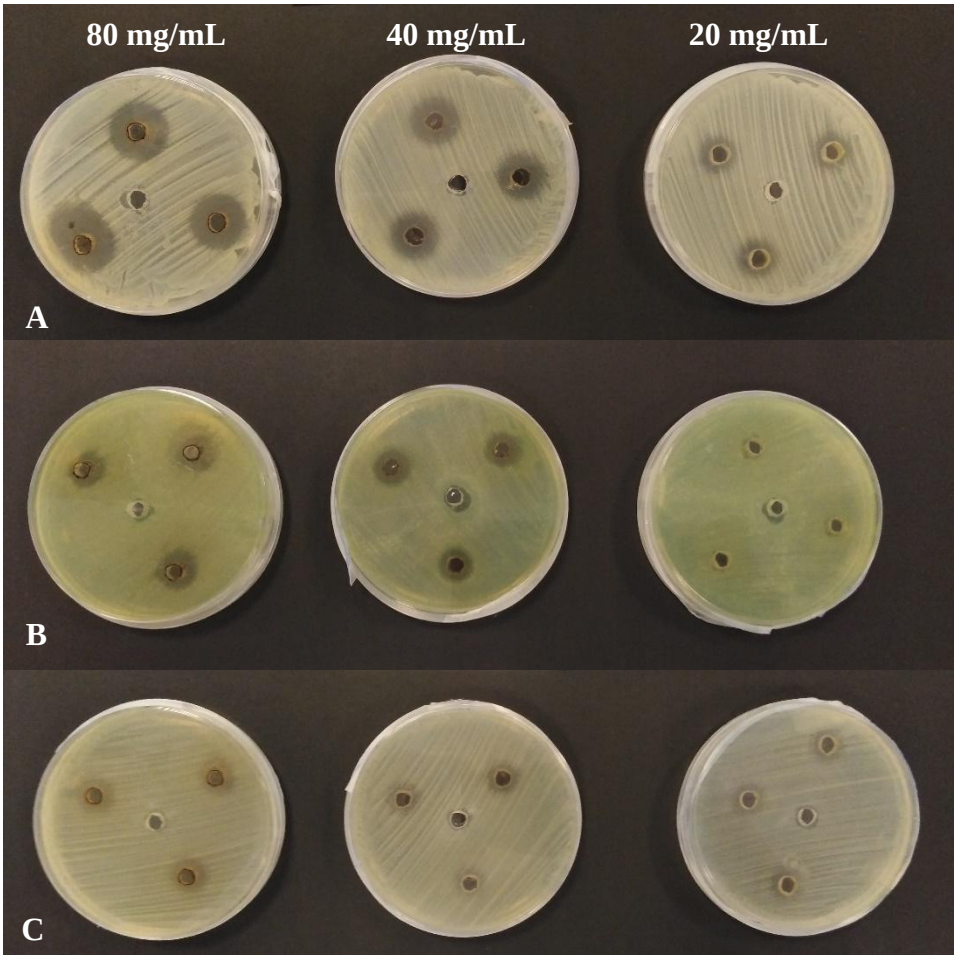
Statistic analysis. To determine the relationship of the antibacterial effect of *O. vulgare* EE on bacterial growth, an analysis of variance (ANOVA) was performed with a significance level of 0.05 and a Tukey test for the comparison of the extracts with the positive control.

Results

Figure 1 shows the results of the antibacterial activity of the EE of *O. vulgare* at concentrations of 80, 40 and 20 mg/mL, against a Gram-positive bacterial species *S. aureus* ATCC 29213, two species of large bacteria Gram-negative such as *P. aeruginosa*

ATCC 27853 and *E. coli* ATCC 25922, it is also observed that Gram-negative strains offer less susceptibility compared to *S. aureus*, in addition, it is possible to observe that ethanol activates the bioactive principles present in the residue dry of *O. vulgare*, being a visible characteristic between the concentrations of the extract and the PC (ethanol).

Figure 1 Antibacterial activity of the crude ethanolic extract of *O. vulgare* against (A) *S. aureus* ATCC 29213 (B) *P. aeruginosa* ATCC 27853 and (C) *E. coli* ATCC 25922



In figure 2, the average of the sizes of the inhibition halos of the EE of *O. vulgare* in the concentrations of 80, 40 and 20 mg/mL is presented, it is observed mean halos of 21.64, 15.24 and 11.45 mm for *S. aureus*, for *P. aeruginosa* of 13.31, 12.27 and 7.35 mm, for *E. coli* 12.5, 11.4 and 10.6 mm, inclusive the antibacterial activity of ethanol is lower compared to the extract.

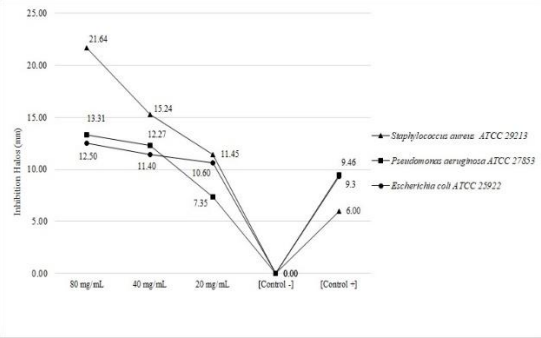
Discussion

The present research work aimed to evaluate the in vitro antibacterial activity of the EE of *O. vulgare* (Oregano).

The results obtained in this study indicate that the EE of *O. vulgare* has antibacterial properties inclusive, greater activity against *S. aureus* ATCC

29213, in relation to *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853, in concentrations of 80, 40 and 20 mg/mL, respectively. According to studies carried out, they have shown an abundance of monoterpenic hydrocarbons and phenolic compounds, the main components being carvacrol, followed by thymol, p-cymene and 1-octacosanol, compounds that have an important broad antimicrobial activity against bacteria, fungi and yeasts[9,31].

Figura 2 Tamaño de los halos de inhibición (mm) de la actividad antibacteriana del extracto etanólico frente a *S. aureus* ATCC 29213 *P. aeruginosa* ATCC 27853 y *E. coli* ATCC 25922



Likewise, the presence of mean halos of: 21.64, 15.24 and 11.45 mm were observed for *S. aureus* ATCC 29213, for *P. aeruginosa* ATCC 27853 of 13.31, 12.27 and 7.35 mm, and for *E. coli* ATCC 25922 of 12.5, 11.4 and 10.6. In other studies, it has been verified that EE had an antibacterial effect against isolated clinical strains with multiple resistance of *S. aureus* with inhibition halos of 10.44 mm, followed by *E. coli* with 9.88 mm and *P. aeruginosa* with 9.77 mm at a concentration of 400 mg/mL[32], with our results, compared to the aforementioned findings, larger halos were obtained, which may be due to the standard ATCC strains used in the aforementioned study, since concentrations of 80, 40 and 20 mg/mL were used for *S. aureus*, *E. coli* and *P. aeruginosa*. Since the *S. aureus* strain presented the highest susceptibility with an inhibition halo of 21.64 mm at the maximum concentration of the extract, it was followed by the *P. aeruginosa* and *E. coli* strains (Figure 1) in our study as in the aforementioned.

Likewise, in another investigation carried out in Iraq, it was reported that said extract of *O. vulgare* at a concentration of 50 and 100 mg/mL presented antibacterial activity against S. aureus with zones of inhibition of 27 - 32 mm., Followed by *E. coli* of 25 - 29 mm and for *P. aeruginosa* of 19 - 28 mm[33], in comparison with our study it is consistent and *O. vulgare* being a medicinal plant with therapeutic potential and the presence of bioactive compounds capable of inhibiting the growth of bacteria.

According to Neira-Llerena[34], with the EE of *O. vulgare* it is shown as the one with the highest antimicrobial activity in this research, after having reached the highest percentages and inhibition halos against S. aureus (16.65 mm) at a concentration of 30 mg/mL, in our study the size of the inhibition halo at a concentration of 80 mg/mL was 21.64 mm, but if we compare it in our study at a concentration of 40 mg/mL it was 15.24 mm in relation to the previous study at a concentration of 30 mg/mL, which is 16.65 mm, the results are very similar, indicating that the antibacterial activity of oregano against *S. aureus* is effective for this microorganism[34].

Regarding its antimicrobial activity, the result of the research confirms that the EE of oregano has antibacterial activity against Gram-positive bacteria such as *S. aureus* and against Gram-negative bacteria such as *E. coli*, *P. aeruginosa*.

However, other studies using the microdilution method reported variability of the MIC of the etha-

nolic extract of *O. vulgare* against *S. aureus* of 500 µg/mL *E. coli* of 250 µg/mL and against *P. aeruginosa* did not present activity[35], Likewise, they also used the essential oil of *O. vulgare* against other clinical microorganisms, having variability in its antibacterial action, the strains of *E. coli* being among the most resistant to antibacterial action of 24.8 to 28.6 µg/mL[36].

The results of this work allow us to conclude that the ethanolic extract of *O. vulgare* is capable of producing compounds with antibacterial potential against nosocomial pathogens, which warrants continuing with the search for the bioactive components responsible for this activity and thus can be used as an alternative therapeutic for the treatment of infections caused by bacteria of clinical importance, as well as its application in food preservation.

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Conflicts of interest

The authors declare that they have no potential conflicts of interest with respect to the research, authorship and / or publication of this article.

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Ethical considerations

The ethical principles of scientific research were respected, as well as the responsible conduct of researchers and the universal principles of biosafety.

Authors' contribution to the article

Orlando Pérez-Delgado, contributed by obtaining the extracts, antibacterial evaluation, statistical analysis and their interpretation. *Rosa Liliana Alvarado-Pineda*, contributed with the writing and interpretation of the results. *Antero Enrique Yacarini-Martínez*, contributed with the writing and analysis of the discussion.

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